
Urinary Tract Infections in Children: Microbial Virulence Versus Host Susceptibility

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Abstract

Urinary tract infections (UTI) are common, dangerous and interesting. This review includes a general background on UTIs and molecular mechanisms of pathogenesis. In addition, we discuss UTI susceptibility and especially the effect of genetic variation on innate immunity.

The symptoms of acute pyelonephritis are caused by the innate immune response and inflammation in the urinary tract decreases renal tubular function and may give rise to renal scarring, especially in childhood. The disease severity is explained by pathogens and their virulence factors triggering signaling through Toll-like receptors (TLRs), interferon regulatory factors (IRFs) and type 1 interferons, and the activation of a host response mediating disease or pathology or clearance of infection. In children with asymptomatic bacteriuria (ABU), in contrast, bacteria persist without causing symptoms or pathology. ABU strains mostly lack virulence factors, and the lack of symptoms has largely been attributed to their lack of virulence. Recently, rapid progress has been made in the understanding of host susceptibility mechanisms. For example, genetic alterations that reduce TLR4 function are associated with ABU while polymorphisms reducing IRF3 or CXCR1 expression are associated with acute pyelonephritis and an increased risk for renal scarring.

Understanding bacterial virulence and host resistance promises new tools to improve the diagnostic accuracy in children with UTI. By combining information on bacterial virulence and the host response, it should be possible to start individualizing diagnosis and therapy. Finally, we propose that the prediction of future disease risk and decisions on prophylaxis and invasive diagnostic procedures might be improved by genetic analysis.

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17.1 Introduction

Bacteria frequently invade the distal urinary tract but in most individuals, infection is not established, due to an efficient antibacterial host defense, reviewed below. However, in patients with voiding dysfunction, malformations and/or molecular immune defects, infection is facilitated, and even bacteria of reduced virulence may establish infection. Most children who develop UTI have a normal urinary tract, and to establish bacteriuria and symptomatic infection, specific virulence factors are required as well as a match with a susceptible host.

Urinary tract infection (UTI) is defined by significant bacteriuria, classically $>10^5$ CFU/ml of urine, but lower counts are considered relevant in a patient with acute symptoms and frequent voiding. Bacteriuria may lead to severe symptoms and even long-term sequelae such as renal scarring, or be asymptomatic and protect against symptomatic disease.

Acute pyelonephritis (APN) is the most severe form of UTI, accompanied by local and systemic inflammation and symptoms. Bacteria establish infection in the renal pelvis and may proceed to invade renal tissues. The patient experiences flank pain, fever >38.5 and general malaise and laboratory tests detect elevated levels of circulating acute phase reactants like CRP, ESR, cytokines and leucocytes. Increasingly used to also quantify local inflammation in the urinary tract are urine cytokine levels, adding much-needed information in addition to pyuria, the classical diagnostic tool to detect inflammation in the urinary tract. Adults with acute pyelonephritis develop bacteremia in about 30 % of cases and urosepsis remains an important cause of death. The frequency of bacteremia or uro-sepsis in children is lower, and, in the pediatric age group, it is not entirely clear to what extent bacteria invade the blood-stream.

Asymptomatic bacteriuria (ABU) is the most common form of UTI, occurring in about 1 % of girls, 2 % of pregnant women and 20 % of both men and women above 70 years of age. These

patients carry $>10^5$ CFU/ml of urine, often for extended periods of time, without developing symptoms. The patients may develop a mild host response that can be quantified as low cytokine levels in urine (IL-8 but not IL-6) as well as variable, low level pyuria and after long-term carriage the patients may develop lymphoid follicles in the bladder mucosa, which resolve after antibiotic therapy. This host response is obviously not strong enough to cause symptoms and the relevance of variable host responses in ABU is unclear.

Acute cystitis is very common, but not well defined in childhood, as diagnosis largely relies on the patient being able to describe the symptoms. In adult women, acute cystitis episodes are exceedingly common, with frequency, dysuria and suprapubic pain and often require antibiotic therapy. In addition, lower tract symptoms may precede upper tract infection, creating a grey zone between patient groups with symptomatic UTI.

The level and severity of UTI is determined by the properties of the infecting strain; mostly *Escherichia coli*. Virulent uropathogenic *E. coli* that cause acute pyelonephritis are carried for extended periods of time in the fecal/perineal flora of highly UTI prone individuals. Invasion of the urinary tract is hindered by an intact urine flow and a highly efficient innate immune response, which senses the presence of invaders and destroys them, so that the urinary tract can regain its "sterility".

UTIs are also a fascinating model, to study the molecular features of host-pathogen interactions at mucosal surfaces. Unlike other mucosal sites that are populated by a complex bacterial flora, the urinary tract is usually infected by a single strain, making analyses of virulence factors and their contribution to human disease quite feasible. Host response variables may also be assessed and directly correlated to disease severity. This chapter focuses on determinants of bacterial virulence and on the host response, especially dysfunctions that tilt the balance towards inflammation, symptoms and disease.

17.2 Pathogenesis of UTI

E. coli strains cause 80–90 % of community-acquired acute pyelonephritis episodes and are especially common in children. Uropathogenic *E. coli* (UPEC) strains are defined by chromosomal virulence genes, which may be activated at different times during infection, to facilitate host tissue attack and bacterial survival. For example, bacterial adhesion to the mucosa is a crucial first step in disease induction, and fimbriae increase tissue exposure to secreted bacterial toxins, like hemolysin and LPS, that damage the tissues by interfering with cellular functions or by directly inducing cell death. UPEC strains can be identified, by typing for virulence factors like P fimbriae.

Critical mechanisms involved in antibacterial host defense have also been identified. Resistance to infection relies on innate immune mechanisms and defects in innate immunity drastically increase UTI susceptibility, both in mice carrying single gene deletions and in patients prone to acute pyelonephritis or ABU. In contrast, specific immunity executed by T lymphocytes and/or B lymphocytes is not involved in acute defense. These findings are consistent with clinical observations, showing that susceptibility to acute pyelonephritis is not associated with hypo-gammaglobulinemia or T lymphocyte deficiencies.

17.3 Bacterial Virulence Factors

17.3.1 Bacterial Adhesion

The ability to adhere to cells in the urinary tract is a critical virulence factor in UTI. UPEC strains express surface fimbriae that recognize specific receptors on host cells. The different fimbrial types are distinguished by their structural features and by the oligosaccharide receptor epitopes that they recognize. Type I fimbriae bind to mannosylated glycoproteins, such as the Tamm-Horsfall glycoprotein, secretory IgA or bladder cell uroplakin. S fimbriae bind sialic acid

epitopes present in sialylated glycoproteins and glycolipids. P-fimbriae recognize Gal α 1-4Gal β -epitopes in the globoseries of glycolipids. The expression of P fimbriae is strongly linked to virulence, being present in about 80 % of strains causing uncomplicated acute pyelonephritis but in <20 % of strains causing ABU. The frequency of P fimbriated strains is reduced in patients with impaired resistance, due to pregnancy, urodynamic defects or genetic abnormalities (see below).

17.3.2 Lipopolysaccharide (LPS)

LPS is the endotoxin of Gram-negative bacteria, containing the membrane anchor Lipid A which is responsible for the toxic effects. Injection of Lipid A mimics many of the symptoms associated with acute pyelonephritis, Gram negative septicemia or other serious Gram-negative infections, including fever, and activates the acute phase response. In addition, LPS carries an invariable oligosaccharide core and a repeating oligosaccharide that determines the O antigen. LPS activates Toll-like receptor 4 (TLR4) signaling, after binding to LBP, CD14 and MD2. This mechanism is essential for innate immune activation during systemic infection but is less important at mucosal surfaces, where the cell-bound CD14 receptor often is missing and MD2 may be weakly expressed.

17.3.3 Capsular Polysaccharide

Capsular polysaccharides are formed from highly repetitive, oligosaccharides and different capsular types are defined by variant antigenic oligosaccharide epitopes. The capsules surround bacteria in blood and tissues and contribute to bacterial survival in the presence of a functional host defense, by preventing lytic effects of complement and antibacterial peptides as well as phagocytosis. Mutant bacteria with altered capsule expression show reduced virulence in experimental UTI models.

17.3.4 Hemolysin

Hemolysins are cytotoxic, pore-forming proteins that permeate the cell membrane, commonly identified by their ability to lyse erythrocytes. Hemolysin production was observed in *E. coli* causing acute pyelonephritis in the 1940s, and hemolysin occurs more often in *E. coli* causing acute pyelonephritis than in fecal isolates even though the difference in frequency is less pronounced than for e.g. P fimbriae.

17.3.5 Metabolic Competition

Most *E. coli* strains causing acute pyelonephritis produce aerobactin, which is an iron binding protein with high affinity, as well as other iron sequestering proteins. By producing such proteins, bacteria compete with the host for nutrients such as iron. Host iron-binding proteins include lactoferrin in mucosal secretions and transferrin in the circulation, and their concentrations increase in response to infection.

17.4 The Innate Immune Response

The innate immune response is activated in a pathogen specific way by P fimbriae mediated adhesion to glycolipid receptors, followed by release of ceramide, activation of TLR4 signaling, and downstream transcription factors like IRF3, which trigger cytokine production and neutrophil recruitment, eventually killing the bacteria. Indeed, the presence of a mucosal cytokine response was first observed in UTI. IL-6 is secreted by uroepithelial cells and acts as an endogenous pyrogen, activates hepatocyte CRP production and stimulates mucosal B cells to produce IgA antibodies. The biological activities of IL-6 thus agree with the clinical presentation of acute pyelonephritis and IL-6 is detected in the blood and urine of patients with acute pyelonephritis.

Infected epithelial cells also secrete IL-8, which is chemotactic for neutrophils. IL-8 forms a gradient across the mucosa and neutrophils migrate towards the gradient, and cross the mucosal barrier and into the urine. The concentrations

of IL-8 correlate with the number of leucocytes in urine, and the chemotactic activity of urine is inhibited by IL-8 antibodies, showing that IL-8 is an essential chemoattractant in UTI. In addition, infection increases the expression of IL-8 receptors, which are important targets for the IL-8 family of chemokines and support neutrophil migration and activation. These mechanisms explain “pyuria”, i. e. the presence of leucocytes in urine, which has been used as a diagnostic tool in UTI since microscopy was first used in the clinic. More recently, a number of additional chemokines have been found in urine, probably reflecting a more complex immune response in the mucosa, especially during long-term bacterial carriage.

These mechanisms also determine the symptoms and signs of acute UTI and several studies have suggested that urine cytokine levels directly reflect the degree of renal involvement.

17.5 Host Factors that Predispose to UTI

The frequency of UTI varies with age, gender and socio-economic factors. UTI morbidity is higher in women than in men, except during the first year and at the end of life. Regardless of gender, certain individuals develop severe, acute infections, some recur and a subset develop a chronic state of tissue damage, leading to renal scarring. For decades, scientists have worked to better understand the factors that determine susceptibility and to develop diagnostic tools to discriminate these individuals from others, who only experience sporadic UTI episodes. Recent studies have started to identify genetic risk factors that distinguish pyelonephritis-prone children from those who consistently develop ABU. Even if the information is limited, it is clear that genetic variation affecting innate immunity influences UTI susceptibility.

Resistance to acute pyelonephritis is also influenced by dysfunctional voiding. Patients with reflux are susceptible to infection with bacteria of lower virulence than patients with anatomically normal urinary tracts. It is not clear if the urine flow, *per se*, is sufficient to maintain

sterility, aided by antibacterial peptides that directly kill the bacteria.

17.5.1 Inflammation and Bacterial Clearance

The inflammatory response is crucial for the antibacterial defense of the urinary tract. Loss of functional Toll-like receptors impairs the innate immune response, and promotes long-term bacterial colonization and ABU. In contrast, defects in the antibacterial effector functions encoded by this pathway, result in severe acute infections, tissue damage and renal scarring.

17.5.2 Toll-like Receptor Signaling and ABU

Children with ABU express lower levels of the TLR4 receptor than age matched controls or children with acute pyelonephritis. They carry mutations in the TLR4 promoter that reduce the efficiency of TLR4 expression. Mice lacking the TLR4 gene also develop ABU, with $>10^5$ CFU/ml of bacteria but no symptoms or tissue damage. Low TLR4 expression and the resulting absence of inflammation can thus be protective rather than destructive.

17.5.3 IRF3, CXCR1 and Acute Pelonephritis

The IRF3 transcription factor enhances the transcription of innate immune response genes including cytokines and chemokine receptors. Mice lacking *Irf3* develop severe acute pyelonephritis with urosepsis and renal abscesses. Mice lacking the IL-8 receptor (*mCxcr2*^{-/-}) also develop severe acute pyelonephritis with urosepsis and surviving animals have renal abscesses and renal scarring. These examples show that single gene deletions affect crucial functions in innate immune signaling, resulting in increased UTI susceptibility in mice. The pathology of the *mCxcr2*^{-/-} was also due to poor neutrophil recruitment and neutrophil activation. Like the

Irf3^{-/-} mice, the *mCxcr2*^{-/-} mice showed acute renal abscesses and surviving mice developed renal scarring.

We also found *IRF3* and *CXCR1* polymorphisms in pyelonephritis prone patients. Children prone to acute pyelonephritis had reduced CXCR1 expression and heterozygous CXCR1 polymorphisms and low mRNA levels suggested that CXCR1 transcription is impaired in this patient group. We also found promoter polymorphisms that reduced promoter efficiency and IRF3 expression in pyelonephritis prone children.

17.5.4 Receptors, Blood Groups and UTI Susceptibility

Uropathogenic *E. coli* colonize mucosal surfaces through adhesion, and thus, resistance to infection can be modified by the repertoire of receptors that the patients express and by soluble, anti-adhesive molecules. Early studies by Stamey et al. proposed that women with recurrent UTI have increased vaginal carriage of uropathogenic bacteria and their uroepithelial cells have since been shown by several groups to have an increased tendency to bind uropathogenic *E. coli*, reflecting an increased density of receptors.

P fimbriated *E. coli* bind to the globoseries of glycolipids, which are antigens in the P blood group system. Through P blood group determination, it is possible to predict the mucosal receptor repertoire and thus, the P blood group can be used as a marker in epidemiologic studies. Individuals of blood group P lack a critical enzyme involved in glycolipid synthesis and lack functional receptors for P fimbriae predicting that they would be more resistant to infection with P fimbriated bacteria. Children of blood group P1 run an increased risk of acute pyelonephritis (about 11 fold) and carry P fimbriated *E. coli* in the intestinal flora, which is a UTI risk factor. Epithelial receptor expression is also influenced by the ABO blood group and individuals expressing globo A are preferentially infected by P fimbriated *E. coli* that recognize a combined Gal α 1-4Gal β and blood group A epitope.

17.5.5 Anti-adhesive Molecules in Urine

Urine contains soluble receptor oligosaccharides or glycoconjugates that bind fimbriae and prevent them from mediating adhesion. The Tamm-Horsfall protein, which is a normal urine constituent, carries terminal mannose residues, that bind type 1 fimbriated *E. coli* and sialic acid residues bind S fimbriae. P fimbriae, which show the strongest disease association, have no known soluble inhibitors in urine.

17.5.6 Specific Immunity and Resistance to UTI

Acute pyelonephritis gives rise to a specific immune response after three to seven days. The mucosal immune response has a relatively short memory, however, and thus most patients have low levels of specific antibodies in urine, when they develop recurrent infections. In experimental studies, mice lacking a specific immune system have surprisingly not shown a drastic increase in UTI susceptibility, and patients with different specific immuno-deficiencies do not primarily develop UTI, suggesting that the specific immune system is not essential for the defense against acute infection.

The main evidence that specific immunity may protect the urinary tract against infection has been generated through vaccination studies. Early studies showed that vaccination with formalin treated whole bacteria markedly increases specific antibody levels. Further studies have used purified antigens (LPS, kapsel, fimbriae, outer membrane proteins) and show that hyperimmunization can protect against infection, provided that the vaccine antigen is expressed by the bacterial strain used for infection and infectious challenge is given while the immune response remains active. The antigenic variation in uropathogenic *E. coli* is a problem for the implementation of vaccination in UTI, however, modern vaccine development gives rise to new optimism. The genetic studies also suggest that genetically

susceptible individuals might be targeted for vaccination.

17.6 Summary

The symptoms of acute pyelonephritis are caused by the innate immune response and urinary tract inflammation decreases renal tubular function and may give rise to renal scarring, especially in children. Antibacterial defence relies on innate immunity and TLR4 signaling initiates this response. Reduced TLR4 function is associated with ABU, while reduced IRF3 or CXCR1 expression is associated with severe acute pyelonephritis and an increased risk of renal scarring.

Most cases of acute pyelonephritis are caused by *E. coli* strains expressing virulence factors that contribute to the establishment of bacteriuria. P fimbriae remain the single virulence factor that best describes the virulence of the infecting strain, but more extensive gene expression methods may also be used to get a more complete picture of the virulence repertoire.

The characterization of bacterial virulence factors and inflammatory profiles may provide new tools for the diagnosis of UTI. Virulent bacteria can, for example, be identified by the expression of P fimbriae. By combining information on bacterial virulence and the host response, it should be possible to start “individualizing” diagnosis and therapy in the large and diverse group of patients with UTI.

References

1. Nielubowicz GR, Mobley HL (2010) *Host-pathogen interactions in urinary tract infection*. *Nat Rev Urol* 7(8):430–441
2. Ragnarsdottir B et al (2011) *Genetics of innate immunity and UTI susceptibility*. *Nat Rev Urol* 8(8):449–468
3. Bhat RG, Katy TA, Place FC (2011) *Pediatric urinary tract infections*. *Emerg Med Clin North Am* 29(3):637–653
4. Montini G, Tullus K, Hewitt I (2011) *Febrile urinary tract infections in children*. *N Engl J Med* 365(3):239–250